Chapter 6

Issues in the risk assessment of the use of microalgae for production purposes

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The use of microalgae for biotechnological purposes has increased rapidly in the past few years. In the United States, oversight of the development of the use of microalgae is included in the purviews of many laws and the regulations that implement those laws. Part of the responsibilities encompassed by these laws is a need to evaluate the risks as well as the benefits from the biotechnology industry. In the United States, efforts to co-ordinate the evaluation of research and the commercialisation of biotechnology, which includes the use of microalgae, have been ongoing since 1986. The recent development of a biofuels and bioproducts component of the biotechnology industry has resulted in new examinations of the roles government agencies play in the oversight of this industry sector. Risk and sustainability assessments for production of microalgae have recently been highlighted by private and government sponsored panels. This chapter discusses the progress of co-ordination and evaluation of such oversight in the United States.

Introduction

Over the past few years, the interest in microalgae for production purposes has grown vastly. In the United States, this is reflected in an increase in industrial activity, and many algae companies are headquartered in the United States. As algae are part of the alternative energy portfolio, their development for industrial use is supported by US government funds for alternative energy, made available by, for instance, the Departments of Agriculture, Energy and Defense. Moreover, algae are seen as industrially useful platforms because in addition to biofuel, they may be used to produce a variety of different products, including commodity chemicals, fine chemicals, food, feed, cosmetics and drugs. Algae are important in biotechnology because they can utilise light energy for growth, but some can also be cultured as heterotrophs, in conventional fermenters.

As regulatory oversight encompassing the algae industry is distributed among several laws in the United States, the harmonisation of risk assessment is part of the United States' interest in algae. Risk and sustainability reviews have been initiated due to mandates of laws requiring oversight or by needs of funding sources. An example of the latter includes a study by the US National Research Council entitled *Sustainable Development of Algal Biofuels in the United States* (Committee on the Sustainable Development of Algal Biofuels et al., 2012), that was supported by the Department of Energy.

Activities of the Algae Working Group of the Biomass Research and Development Board

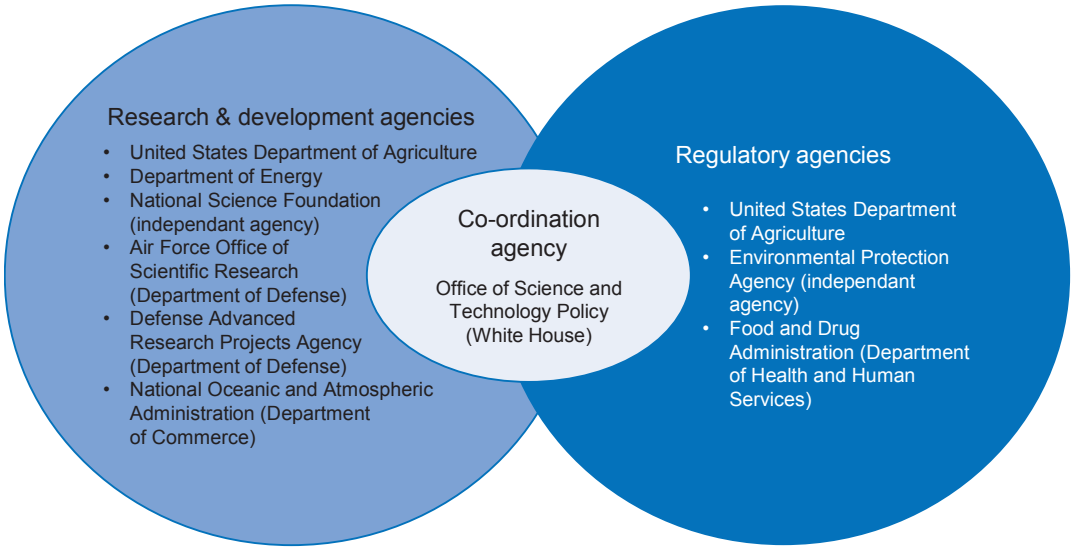
The Algae Working Group (AWG) is one of the support units of the Biomass Research and Development Board (BRDB), and is currently comprised of about 20 members from 8 departments or independent agencies (Figure 6.1). The scope of the AWG extends beyond the BRDB's needs, and includes topics such as research and regulation and other-than-energy interests, e.g. food, cosmetics, agriculture and the environment. The mission of the AWG, as described in 2012 was: advise, communicate and co-ordinate federal research, development, demonstration and deployment activities relating to the production and use of algae and their products/co-products in a sustainable manner within an appropriate regulatory framework.

Topic areas of the AWG in 2011 were: sustainability, algae biology and production, algae harvesting and extraction. Current topics include assessing the scope of oversight responsibilities within the US federal government and designing and developing an algae information resource on the aspects of algal technology, including information on the attributes of specific algae, descriptions of research and regulatory responsibilities, links to public resources of information, links to public information on research funding, and relevant event calendars. An overview of the AWG's activities and participation is presented in Table 6.1.

As noted, a variety of laws and regulations apply to the algae biotechnology industry in the United States. This results in a situation where regulatory oversight of algae is distributed among many statutes according to uses, such as foods, drugs and cosmetics, agriculture, or occurrences such as harmful algal blooms. Some examples of applicable regulatory legislation are the Food Safety Modernization Act (FSMA, 2011), the Toxic Substances Control Act (TSCA, 1976) and the Clean Water Act (CWA, 1972). To help resolve overlap of responsibilities, in 1986, most regulatory agencies involved with

biotechnology jointly described their oversight functions and agreed to lead responsibilities described in the Office of Science and Technology Policy's (OSTP) Coordinated Framework for Regulation of Biotechnology. However, some laws apply to all of the US federal government. This is the case for the National Environmental Policy Act (1970).

Figure 6.1. **The interagency Algae Working Group**



Source: The Interagency Algae Working Group/Biomass Research and Development Board (U.S.A.)

Table 6.1. **Overview of the Algae Working Group's activities (2012) and participation**

FY 2012 activities	Federal participants
– "Most/least wanted" algae list: genera of algae that are of particular interest as research models, production strains; also, algae strains that are problematic (invasive, toxic) – Scope of agency activities summarising agency mission areas and objectives related to algae – Working group report of activities: topical white papers resulting from meeting discussions, findings of knowledge gaps and descriptions of any other collaborative activities	Joyce Yang, Department of Energy/OBP Mark Segal, Environmental Protection Agency/OPPT Co-chairs Participating agencies and departments: – National Science Foundation – Food and Drug Administration (Department of Health and Human Services) – United States Department of Agriculture – Department of Energy – National Oceanic and Atmospheric Administration (Department of Commerce) – Department of Defense/Air Force Office of Scientific Research – Department of Defense/Defense Advanced Research Projects Agency – Environmental Protection Agency

Source: The Interagency Algae Working Group/Biomass Research and Development Board (U.S.A.)

Example: The Toxic Substances Control Act (TSCA)

Oversight of industrial and commercial chemicals production is provided by the Toxic Substances Control Act, with specific implementation for biotechnology micro-organisms provided by Microbial Products of Biotechnology; Final Regulation

under the Toxic Substances Control Act,[1] referred to simply as the TSCA, Biotechnology Rule (1997). This legislation applies to micro-organisms (including all types of algae) that are manufactured, imported or processed for commercial activities, including research and development (R&D) activities, that are considered "new", and describes the pre-manufacturing (MCAN) review requirements. "New" in this context means those that are not on the TSCA's Inventory of Chemical Substances. Examples of algae commercial applications covered by this regulation include biofuels, the production of specialty and fine chemicals and biofertilizers. New micro-organisms are defined as those comprised of genes from different genera and/or with chemically synthesised genes. Pre-manufacturing review of R&D (TERA) for the micro-organism is required if the micro-organism is not contained within a structure. Some chemical products of algae may be "new" chemicals, requiring pre-manufacturing (PMN) review of the chemicals.

Issues identified by the United States Environmental Protection Agency

The following issues were identified and presented in a 2009 EPA workshop:

- Environmental exposure from algae biofuel production under various levels of containment:
  - integrity of modern algae photobioreactors (e.g. plastic bags, other)
  - releases of algae for ponds (intentional and accidental)
  - environmental exposure under normal production:
    - dispersal by aerosols
    - dispersal by wildlife (birds, insects, reptiles, terrestrial animals
  - environmental exposure under catastrophic failure of containment systems.

Participants were asked to express why and when the listed scientific information is needed. The simple answer for "Why?" was that a scientifically credible risk assessment required that these kinds of data and information be available for evaluations, and that science-based risk hypotheses be taken into account, that are falsified based on high-quality scientific information that is useful for risk assessment. They also determined that there was an immediate need for this scientific information since, even in 2009, it was acknowledged that dozens of companies were currently operating with naturally occurring micro-organisms, and the use of genetically engineered strains by companies that were considering their commercialisation was on the horizon.

In another forum,[2] additional insight was provided by expanding on the topics identified in the 2009 workshop focusing on specific information needs as follows:

- Technology issues, e.g.: release potentials vary, depending on the design of the reactors used. Some design features may have positive or negative effects depending on the specific conditions:
  - open raceway ponds vs. closed photobioreactors vs. hybrid designs
  - inputs for production:
    - water use – freshwater, saline, brackish, wastewater, etc.
    - nitrogen and other nutrients
    - use for wastewater clean-up and CO_2 sequestration

- siting issues, e.g.:
  - the consequences of releases, when they occur, vary depending on the ecosystem in which the production facility is located, e.g. desert, coastal regions, surface freshwater, agricultural areas, urban regions.

In addition, other topics were noted:

- Human health and ecological effects, e.g.:
  - releases of algae into the environment:
    - ❖ phycotoxin production
    - ❖ propensity for blooming/anoxia
    - ❖ effects on food web by substitution of preferred food source (native algae) with dominant supply of alternate (escaped) algal species and/or different lipids produced by those algae)
    - ❖ stress-induced production of potentially bioactive biofuel molecules in the environment under commonly found nutrient-limited conditions
    - ❖ competition with indigenous species
    - ❖ dispersal in the environment
    - ❖ gene transfer from transgenic algae
  - release of wastewater and waste biomass, e.g.:
    - ❖ introduction of biological materials, chemicals, nutrients, additives (e.g. from flocculation) into the environment
    - ❖ bioaccumulation of heavy metals from industrial sources of CO_2.

Finally, other progress in identifying assessment issues for algae has taken a different track. A third workshop,[3] on assessing a new paradigm for risk assessment of micro-organisms designed using synthetic biology, was held in 2011. Participants were experts from multiple disciplines, who addressed how to perform risk assessments for micro-organisms produced by synthetic biology. The issues identified at this workshop are common to many algae biotechnology applications. To help identify the key issues, an example was used of a cyanobacterial species designed to produce a commodity chemical. Table 6.2 presents the results of the workshop-research needs, where the participants generated a summary of five main research categories for environmental risk assessments of synthetic biology applications.

Conclusion

Some items in the discussion on environmental risk assessment of genetically engineered algae may demand a special focus.

Familiarity with key algal species

While there is a some familiarity with a number of key species that have already been used extensively in actual production and that may serve as a baseline for assessment, for many species, little is known about their roles in the environment, and thus extrapolation from observations under culture to conditions expected if released from culture is difficult.

Table 6.2. **Summary of five main research categories for environmental risk assessments of synthetic biology applications**

Research category	Specific questions	Reasons given by participants
Rates of evolution and changes in functionality	– Investigate the rate of evolution for changes in functionality.	(Not given a high priority, and therefore no reason given)
Survival and persistence of the organism	– Is the organism compatible with the environment and other populations? – Can the organism survive in a dormant or resting state? – What is the "fitness cost" of the engineered gene and how much of a fitness cost would encourage rapid fall off or "extinction" of the organism in the wild? – How many survival competition tests are needed? Studies should include a whole community analysis, under a variety of environmental conditions. – Consider everyone (e.g. the grazers), not just the competitors.	– Encapsulates the genetic history of the organism and useful in understanding its evolution. – Companies are not expected to do a lot of work in this area; this information is difficult to come by, but important.
Fate and transport of functional genetic material	– Ability of DNA to persist after death? – Which (groups of) organisms may acquire the gene? – Does the target gene remain functional in other hosts? – In what ways can the target gene alter existing genomes? – Introduce fragments of the introduced cassette and measure what is picked up by other micro-organisms.	– As the general public would be very interested in this, a risk assessment would certainly need to cover this. – Fills in gaps, leads to useful information for both regulation and the development of organisms. – But it is also the subject that is least understood of what was talked about in the workshop, and therefore most interesting. – Most relevant from the policy perspective. – A risk we do not understand. – Limiting fate of genetic material.
Physiological differences and differences in functionality between the wild and novel organism	– What is the natural risk of these wild organisms (baseline considerations)? – How do we compare the additional risk due to novel genes? – Investigate secondary metabolites. How many should we look at and at what concentrations? – What are cells doing on a daily basis? Have they changed? Are they the same cells you started with? Are they behaving as desired? – Generate a profile of how the genome and the products of the cell are changed by the addition of engineered genes.	– Captures a broad understanding of the organism before it is modified and allows the modified organism to be compared with a baseline. – By focusing on this category, issues contained in research categories 1 and 2 would be addressed. – This is a "need to know" before it can be said whether the new organism will change ecosystems. – This category has the least amount of available data. – This represents the hazard part of the risk assessment which is important. – This will be the trigger of regulation. – This information is important for the first step for the risk assessment and will temper what questions to ask in other areas.
Probabilistic modelling of gene transfer	– Can modellers guide the parameters and data needed to predict gene uptake? – Would a model separate naturally occurring genes prevalent enough to assume that they have been thoroughly sampled throughout evolution from ones that are rare be useful? Can we create a threshold of exoticism for genes to guide us?	(Not given a high priority, and therefore no reason given)

Familiarity with a variety of existing production facility designs

Production facility design is undergoing rapid evolution. For some types of design, each manufacturer has developed an approach that may be unique for its needs. Much experience with these designs is proprietary. While experience with traditional open pond designs of the raceway approach is significant, those that involve advance photobioreactor or other non-traditional designs have little history of safe use. Thus, for both traditional and advanced facilities, an analysis should be made about their probability of failure, based on existing experience.

It may be expected that the production technology will develop rapidly with increasing success and needs. This includes both facility and organism design. It may be expected that new techniques will evolve for the genetic engineering of algae in order to make production more efficient. A thorough understanding of the effects of these technical advancements on potential risks associated with their use needs to be established concomitantly with the understanding of the effects of the advancements on improvements in production.

Notes

1. www.epa.gov/fedrgstr/EPA-TOX/1997/April/Day-11/t8669.htm.
2. Presented to the National Research Council Committee for Sustainable Development of Algal Biofuels, 17 March 2011.
3. "Comprehensive Environmental Assessment and Synthetic Biology Applications", held at the the Woodrow Wilson International Center for Scholars, Washington DC, in July 2011.

References

Committee on the Sustainable Development of Algal Biofuels, Board on Agriculture and Natural Resources, Board on Energy and Environmental Systems, Division on Earth and Life Studies, Division on Engineering and Physical Sciences National Research Council (2012), *Sustainable Development of Algal Biofuels*, The National Academies Press, Washington DC.